THE REJECTION OF CLAIMS 1-87 UNDER 35 U.S.C. §103

Claims 1-87 are rejected under 35 U.S.C. §103(a) as being unpatentable over Khrapko *et al.* (J. DNA Sequencing and Mapping 1: 375-388) in view of Drmanac *et al.* (DNA and Cell Biology 9: 527-534) because Khrapko *et al.* teaches the use of a probe array comprising a constant region attached to a solid phase and a variable region and Drmanac *et al.* teaches the use of a probe array having three random positions. It is asserted in the Office Action that the "claims are not limited to any specific length of constant and random regions nor any minimum number of probes" so that the claims read on the use of as few as two probes of any length having a variable region of a single nucleotide. It is alleged that because Khrapko teaches the use of a probe array comprising a constant region attached to a solid phase and a variable region of 1-2 nucleotides, and Drmanac teaches the use of probes of 11-20 nucleotides and 3 random positions, the only difference between Khrapko *et al.* and the instant claims is the use of "labels and solid phases." The Examiner concludes that since only the interaction between the probe and target is allegedly critical in the method, any labels or solid phase material can be selected.

It is also asserted that although some of the claims differ from Khrapko *et al.* in the recitation of identification and detection steps, Drmanac teaches a method of nucleic acid detection or identification comprising contacting a nucleic acid with a sample bound to a solid phase. It is concluded that it would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to use the materials of Khrapko *et al.* in the method of Drmanac because the methods of Drmanac *et al.* increase the discrimination of detection methods, an advantage explicitly taught as desirable in Drmanac *et al.*.

The rejection is respectfully traversed.

Relevant law

In order to set forth a prima facie case of obviousness under 35 U.S.C. §103: (1) there must be some teaching, suggestion or incentive supporting the combination of cited references to produce the claimed invention (ACS Hospital Systems, Inc. v. Montefiore Hospital, 732 F.2d 1572, 1577, 221 USPQ 329, 933 (Fed. Cir. 1984)) and (2) the combination of the cited references must actually teach or suggest the claimed invention. Further, that which is within the capabilities of one skilled in the art is not synonymous with that which is obvious. Ex parte Gerlach, 212 USPQ 471 (Bd. APP. 1980). Obviousness is tested by "what the combined teachings of the references would have suggested to those of ordinary skill in the art" In re Keller, 642 F.2d 413, 425, 208 USPQ 871, 881 (CCPA 1981), but it cannot be established by combining the teachings of the prior art to produce the claimed invention, absent some teaching or suggestion supporting the combination (ACS Hosp. Systems, Inc. v Montefiore Hosp. 732 F.2d 1572, 1577. 221 USPQ 329, 933 (Fed. Cir. 1984)). "To imbue one of ordinary skill in the art with knowledge of the invention in suit, when no prior art reference or references of record convey or suggest that knowledge, is to fall victim to the insidious effect of a hindsight syndrome wherein that which only the inventor taught is used against its teacher" W.L. Gore & Associates, Inc. v. Garlock Inc., 721 F.2d 1540, 1553, 220 USPQ 303, 312-13 (Fed. Cir. 1983).

The prior art must provide a motivation whereby one of ordinary skill in the art would have been led to do that which the applicant has done. Stratoflex Inc. v Aeroquip Corp., 713 F.2d 1530, 1535, 218 USPQ 871, 876 (Fed. Cir. 1983). In addition, the mere fact that the prior art may be modified in the manner suggested by the Examiner does not make the modification obvious unless the prior art suggests the desirability of the modification. In re Fritch, 23 USPQ 1783 (Fed. Cir. 1992).

Also, it is impermissible to ignore the advantages, properties, utilities and unexpected results that flow from the claimed invention; they are part of the invention as a whole. In re Sernaker, 702 F.2d 989, 217 USPQ 1 (Fed. Cir. 1983). Unexpected properties must always be considered when determining obviousness. A compound's structure and properties are inseparable so that unexpected properties are part of the subject matter as a whole. In re Papesh, 315 F.2d 381, 137 USPQ 43 (CCPA 1963).

The claims

Claims 1-55, 58-60, and 63-77 are directed to methods of sequencing a target nucleic acid molecule by hybridizing an array of probes that contain a variable region to fragments of the target nucleic acid and then determining the molecular weight of the members of the resulting hybridized array. Claim 56 and claims dependent thereon recite that the probes also include a double-stranded region.

Claims 124-126 are directed to an array of probes that contain a variable region on a solid support that includes matrix for mass spectrometry. Claim 124 recites that there are 4R probes, where R is the length of the variable region. Claim 125 specifies that a probe includes a mass-modifying functionality. Claim 126 recites that the probes include a single-stranded and a double stranded region, where the variable region is of length R. Claim 127 is directed to directed to a system that includes the array of claim 124, a mass spectrometer and a computer.

Differences between the teachings of the cited references and the claimed subject matter

Khrapko *et al.*

Khrapko *et al.* describes a technique of DNA sequencing by hybridization with an oligonucleotide matrix (SHOM) and experiments to test the method on a short (17 nucleotides) DNA fragment. he method relies upon hybridization of a **labeled** fragments of a target sequence to a set of 65,536 (4^8) oligomers of 8

nucleotides long, which constitute all possible combinations of 8-mers. The sequence of a particular target can be resolved by identifying the oligonucleotides to which it hybridizes and comparing overlaps among the hybridizing oligomers. The efficiency of the method is stated to depend on the ability to sort out effectively perfect duplexes from imperfect duplexes (i.e., containing base pair mismatches), which can be achieved by comparing the temperature-dependent dissociation curves of the duplexes formed by DNA and each of the immobilized oligonucleotides, with standard dissociation curves for perfect oligonucleotide duplexes to thereby identify perfect duplexes and/or the degree of mismatch. Hence the method requires a determination of the dissociation curve for all hybridizing oligonucleotides.

Prior to embarking on the task of developing dissociation curves for 65,536 perfect octamers, the method was tested on a model heptadeca-nucleotide. In experiments described in Kharpko *et al.* to test SHOM, single-stranded 8-mers were immobilized to a polyacrylamide-covered glass plate. Four single-stranded 17-mers differing by a single base substitution were separately hybridized to the immobilized 8-mers, each of which was complementary to a portion of one of the 17-mers. The hybridizations would thus form perfect as well as imperfect (single mismatches) duplexes. The duplexes were subjected to a series of washes at increasing temperatures and thermal dissociation curves were generated and compared to distinguish perfect from imperfect hybrids to thereby identify the perfectly matched hybrids from which the sequence of the target could be deduced.

It is suggested in Khrapko *et al.* that additional continuous stacking hybridization (CSH), referred to as hybridization of DNA with immobilized octanucleotides **in the presence of** labeled selected pentanucleotides to form a continously stacked perfect duplex of 13 base pairs, could increase the fidelity of SHOM (emphasis added, see page 376, first full paragraph in left column). Additional experiments reviewed in Khrapko *et al.* include a "numerical"

experiment to estimate the efficiency of CSH. In the description of CSH (p. 385, first full paragraph in left column), Khrapko *et al.* states that it is based on the fact that when two oligonucleotides are **simultaneously** hybridized to a longer one, the two duplexes are mutually stabilized if they are positioned side-by-side due to a stacking contact between them. Figure 8 of Khrapko *et al.* is said to illustrate this effect.

Figure 8 of Khrapko *et al.* shows dissociation curves for four different hybridization products. In the four hybridization reactions, a ^{32}P-labeled 5-mer and the "test" 17-mer were simultaneously hybridized with an immobilized oligonucleotide (i.e, four different oligonucleotides were immobilized on matrix: 3 different 8-mers and one 7-mer). The hybridization products were subjected to washes of increasing temperature in order to generate the dissociation curves shown. It is concluded that (1) the 5-mer makes a stable duplex when hybridized to a complementary 17-mer together with immobilized 8-mer due to the continuous stacking contact and (2) the stability of the 5-mer duplex decreases if stacking is disrupted by nucleotide displacement, gap or terminal mismatch.

Khrapko *et al.* teaches that sequencing is effected by identifying hybrids by detecting the labeled oligonucleotide and determining the pattern of sequenced hybrids. Khrapko *et al.* does not teach or suggests determining the sequence by determining the molecular weights of the probes in the hybridized target array.

Drmanac *et al.*

Drmanac *et al.* describes experiments designed to investigate possible DNA hybridization conditions that may permit discrimination between perfectly matched duplexes and duplexes with a single mismatch. In these experiments, single-stranded DNA was spotted on a membrane and then hybridized with an oligomer probe end-labeled with ^{32}P. Autoradiographs of the filters were made and, for discrimination measurements, the dots were excised from the dried

filters after radiography, and the radioactivity of the dots was measured using liquid scintillation counting methods. Preliminary characterization of the thermal stability of short oligonucleotide hybrids was determined on prototype fully matched hybrids or hybrids containing one mismatch: (1) TGCTCATG or GCTCAT hybridized to dot blots containing NCATGAGCANN and (2) GCTCAT hybridized to dot blots of NNCATGAGTTN.

In addition to experiments with model oligonucleotides, an M13 vector and derivative thereof (i.e., vector IF which is an M13 recombinant with a 921-bp human interferon gene insert that carries a single perfectly matched target) were used as a system for a practical demonstration of hybridization to short oligonucleotide probes of 6, 7 or 8 nucleotides. It is concluded in Drmanac *et al.* that using low-temperature conditions, sufficient difference in hybridization signal was obtained between the dot contaning the perfect and mismatched targets and the dot containing only the mismatched targets.

To allegedly show the general utility of the proposed conditions, Drmanac *et al.* examined hybridization of 4 heptamers, 10 octamers and 14 additional probes up to 12 nucleotides long in the M13 system. To allegedly show the utility of the method in fingerprinting unknown clones for the presence of a short sequence, three probes 8 nucleotides long were tested on a collection of 51 plasmid DNA dots made from a library in Bluescript vector.

Drmanac *et al.* does not teach or suggest determining a sequence by determining the molecular weights of the probes in the hybridized target array.

The Office Action fails to establish that the claims are *prima facie* obvious

Relevant Law

In order to set forth a case of *prima facie* obviousness under 35 U.S.C. §103, the differences between the teachings in the cited reference must be evaluated in terms of the whole invention, and the prior art must provide a teaching or suggestion to the person of ordinary skill in the art to have made the changes that would produce the claimed product. *See, e.g.,*

Lindemnann Maschinen-fabrik Gmbh v. American Hoist and Derrick Co., 730 F.2d 1452, 1462, 221 U.S.P.Q.2d 481, 488 (Fed. Cir. 1984). The mere fact that prior art may be modified to produce the claimed product does not make the modification obvious unless the prior art suggests the desirability of the modification. *In re Fritch*, 23 U.S.P.Q.2d 1780 (Fed. Cir. 1992); *see, also, In re Papesh*, 315 F.2d 381, 137 U.S.P.Q. 43 (CCPA 1963).

Analysis

The combination of cited references does not result in the instantly claimed methods, arrays or systems

Claims 1-55, 58-60, and 63-77, as well as claims 88-123, each require the determination of the molecular weights of the nucleic acids of the target array. Claims 124-126 are directed to arrays containing probes on a solid support that comprises matrix material for mass spectrometric analysis, and claim 127 is directed to a system that contains the array, a mass spectrometer, and a computer.

Neither Khrapko *et al.* nor Drmanac *et al.*, singly or in any combination thereof, teaches or suggests a method of sequencing that includes a step in which the molecular weight of hybridized targets is determined. The methods rely upon the use of a label, such as a radiolabel to detect the pattern of hybridization. None of the references, teaches or suggests elimination of the labels and pattern determination by using mass spectrometry to identify and detect the hybridized target and to thereby determine the sequence of the target. For mass spectrometry, labels are not used, since detection is effected by virtue of the molecular weight of the molecules and constituent groups and atoms thereof. There is no suggestion in either reference to eliminate the label, which is an essential component of the methods. Hence the instantly claimed methods are neither taught nor suggested by the cited references, singly or in any combination thereof.

Claim 56 and claims dependent thereon, further specify that the probes contain a double-stranded region and a single-stranded region, which contains the variable sequences. Hence, in methods of these claims, the target nucleic acid is hybridized to an array of probes that include a double stranded portion. Neither Khrapko *et al.* nor Drmanac *et al.*, singly or in any combination thereof, suggest a method in which the **target** nucleic acid is hybridized to an array of probes that contain a double-stranded nucleic acid and single-stranded nucleic acid. In the methods of these references, the target is hybridized to single-stranded probes.

In the CSH method described by Khrapko *et al.* states that it is based on two oligonucleotides are **simultaneously** hybridized to a longer single-stranded one, the two duplexes are mutually stabilized if they are positioned side-by-side due to a stacking contact between them. This is different from hybridization the target oligonucleotide to a probe that is partially double-stranded. No suggestion for modification of this method is taught or suggested. Neither reference teaches, suggests nor provides any motivation to have modified its method by creating probes with a double- stranded region.

Similarly, the combination of teachings of the cited references provides no suggestion for the preparation of arrays of probes that are immobilized on a solid support that includes matrix material for mass spectrometry (claims 124-126), nor an array that contains 4^R immobilized probes that contain a double-standed portion and a single-stranded portion that includes a variable region of length R (claim 124). Since neither reference mentions or suggests anything about mass spectrometry, there is no teaching or suggestion for inclusion of matrix in the support on which probes are immobilized.

Therefore, the Examiner has failed to set forth a prima facie case of obviousness.

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In view of the amendments and remarks herein, reconsideration and allowance of the application are respectfully requested.

Respectfully submitted,
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